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# Improvement of Pharmacokinetics and Antitumor Activity Against Human Hepatoma Cell Line by Using Adriamycin-Entrapped Stealth Liposomes

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Preferential accumulation in the reticuloendothelial system is one of the major obstacles to the use of liposomes as a drug carrier for targeting therapy. To reduce their uptake, ganglioside GM1 was introduced into the components of conventional liposomes that had been used in our targeting experiments. Two types of such liposomes were prepared. Tissue distribution studies on Adriamycin entrapped in both types of liposomes clearly indicated that the uptake of Adriamycin by liver and spleen decreased to the level comparable to that of free Adriamycin administration. By contrast, the level of Adriamycin in the serum remains high, and some increase was observed in the accumulation to the tumor. Furthermore, Adriamycin in these liposomes, which were conjugated with anti-α-fetoprotein (AFP) antibody, inhibited the growth of AFP-positive human hepatoma Li-7 more efficiently than free Adriamycin or Adriamycin in antibody-conjugated conventional liposomes. 

KEY WORDS: **stealth liposomes, GM1, targeting chemotherapy, hepatoma**

## INTRODUCTION

Recently liposomes have attracted much attention as a drug delivery system to promote the delivery of anticancer agents to the target tumors [1,2]. Liposomes, synthetic biomembranes primarily composed of phospholipids, are a kind of microcapsule capable of encapsulating the anticancer agents without chemical modification. Furthermore, by conjugating a tumor-related monoclonal antibody to the surface of liposome, efficient targeting chemotherapy can be performed for tumors that express tumor antigens such as carcinoembryonic antigen (CEA) and α-fetoprotein (AFP) [3].

However, the delivery system with liposomes has also some serious disadvantages. Especially, liposomes have a tendency to be readily entrapped by the reticuloendothelial system (RES), such as Kupffer cells in the liver and the splenic macrophages. Thus, liposomes are rapidly removed from the blood circulation; this prevents anticancer agents from reaching the tumor. By reducing their size, the number of liposomes taken up by the RES can be decreased to some degree [4], but its effectiveness is still limited. In this study, therefore, we changed the lipid composition and prepared so-called "stealth liposomes," to evade uptake by the RES. With these liposomes, we evaluated the possibility of pharmacokinetic improvement and reinforcement of antitumor effect by using human hepatoma in nude mice as a model system.

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## MATERIALS AND METHODS

### Chemicals

Egg yolk phosphatidylcholine (egg PC) was a gift from Nippon Fine Chemical Co. (Osaka, Japan). Cholesterol (Chol), sphingomyelin (SM), dipalmitoylphosphatidylethanolamine (DPPE), and dipalmitoylphosphatidic acid (DPPA) were obtained from Sigma Chemical Co. (St. Louis, MO). 3-(2-pyridyldithio)propionyl-dipalmitoylphosphatidylethanolamine (DTP-DPPE) was prepared by reacting N-hydroxysuccinimidyl3-(2-pyridyldithio)-propionate (SPDP) with DPPE as described by Barbet et al. [5]. Ganglioside GM1 was provided by Sumitomo Pharmaceuticals Co., Ltd (Osaka). SPDP was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), and a stock solution was made in ethanol at a concentration of 20 mM and stored at −20°C. Dithiothreitol was purchased from Sigma Chemical Co. and dissolved at 500 mM in water. Adriamycin (ADM) was used as an antitumor agent and was obtained from Kyowa Hakkou Kogyo (Tokyo).

### Preparation of ADM-Encapsulated Liposomes

The lipids dissolved in an organic solvent (chloroform/methanol: 2:1) were mixed in a pear-shaped flask at various molar ratios as described below, and the organic solvent was evaporated at 45°C by rotation with a vacuum evaporator to prepare thin lipid films. The lipid films were mixed with 1 ml of ADM solution (20 mg/ml in water); by vortex dispersion, ADM-encapsulated liposomes (Lip-ADM) were prepared. These multilamellar vesicles were then prepared into small unilamellar vesicles by ultrasonic treatment with a sonicator (type UCD-110, Japan Biotech, Tokyo). Unencapsulated ADM was removed by gel filtration on a Sephadex G-50 column.

For the present studies, we prepared three types of liposome with different lipid composition. Lipid composition of conventional Lip-ADM, which has been used in our previous experiments [2,3], and was used as a control in this research, was egg PC/Chol/DPPA/DTP-DPPE: 10:5:1:0.16 in molar ratio. The major components of the conventional Lip-ADM were egg PC and Chol, which have a stabilizing effect on the bilayer. DPPA, a negatively charged phospholipid, was added in order to increase the incorporation of ADM into the liposome. DTP-DPPE served as a bridge to bind liposome and antibody. On the other hand, two types of improved liposome were prepared by adding ganglioside GM1 to the composition of lipids. We call these stealth liposomes (st.Lip-ADM). In type (I) stealth liposome (st.Lip-ADM(I)), DPPA was removed from the conventional Lip-ADM and instead GM1 was added at 7.5% of egg PC (in molar ratio, egg PC/Chol/GM1/DTP-DPPE: 10:5:0.75:0.16). In type (II) stealth liposome (st.Lip-ADM(II)), one-half of egg PC was replaced by SM to increase the rigidity of liposome (egg PC/Chol/SM/GM1/DTP-DPPE: 5:5:5:0.75:0.16). The size of liposome was determined by a dynamic laser scattering method using ELS-800 (Ohtsuka Densi, Osaka).

### Preparation of Antihuman AFP Monoclonal Antibodies

Antihuman AFP monoclonal antibody (Ab) was obtained from hybridoma by fusion of a mouse myeloma cell line (P3-U1) and splenic cells of BALB/c mouse immunized with AFP derived from human placenta [6]. The antibody 19-F-12 (IgG2b) with the highest affinity for AFP-producing human hepatoma cell line was used in the experiments. The antibodies were collected from mice in the ascites form and purified by passage through a protein A–Sepharose 4B (Pharmacia) column.

### Conjugation of the Monoclonal Antibody to the Liposome Surface

The antibody was conjugated to liposomes as described previously with a slight modification originally described by Barbet et al. [5]. The antibody (1–2 mg/ml) was mixed with SPDP to give a final concentration of 0.1 mM for 30 min at room temperature and then transferred to acetate buffer (0.1 M, pH 4.5, 0.145 M NaCl) by gel filtration through a Sephadex G-50 column. Protein-bound dithiopyridine was treated with 50 mM dithiothreitol for 40 min at room temperature and eluted through a Sephadex G-50 column with an acetate buffer solution. The free thiol-bearing protein thus activated was immediately mixed with the liposome suspension. After adjustment of pH to 8.0 with 1 M sodium borate, the mixtures were allowed to react at room temperature for 24 hr.

### Tumor Cells and Animals

The human AFP-positive hepatoma cell line, Li-7, was a gift from the National Cancer Center (Tokyo). It was maintained by serial transplantation to the backs of nude mice. Prior to the experiment, tumor tissues were sliced into blocks about 3 × 3 mm in diameter and transplanted subcutaneously to the back side of female BALB/c *nu/nu* mice (body weight about 20 g) (Nippon Clea Co., Tokyo). Tumors were allowed to grow until the estimated tumor weight (calculated as $1/2 \times \text{length} \times \text{width}^2$) [7] reached about 250 mg. Female B10.BR mice (body weight about 20 g) were also used in pharmacokinetic study.

### Tissue Distribution Studies on ADM

Various forms of ADM were injected via the tail vein of B10.BR mice (experiment 1) and tumor-bearing nude mice (experiment 2) in a single dose equivalent to to 5 mg/kg (experiment 1) or 10 mg/kg (experiment 2) of ADM. At 1, 4, 8, and 16 hr after the injection, blood was collected from the retro-orbital venous plexus under ether anesthesia. Various organs, including liver, spleen, heart,

and tumor, were excised immediately and weighed. The organs were homogenized in 5 ml of 0.3 N HCl–50% ethanol with a high-speed mixer (Ultradisperser LK21, Yamato Scientific CO., Tokyo). The homogenates were centrifuged at 20,000*g* for 20 min at 4°C. In the case of liver, a portion (about 100 mg) was used for homogenization; in our preliminary experiments, concentrations of ADM were not different from portion to portion of the liver. The ADM concentration in the supernatant was measured by fluorophotometry (excitation wave = 490 nm; emission wave = 590 nm) (Hitachi F3010, Tokyo). Tissue homogenates from nontreated mice were prepared by the same procedure; the values thus obtained were regarded as background and were subtracted from those of treated mice.

### Antitumor Effects of ADM in Liposomes *In Vivo*

As described above, Li-7 was transplanted to the backs of nude mice. When the estimated tumor weight had reached approximately 250 mg, mice were randomly divided into the following five groups: (1) saline (control), (2) free ADM, (3) conventional Lip-ADM = Ab, (4) st.Lip-ADM(I) = Ab, and (5) st.Lip-ADM(II) = Ab. Each group of animals received, through the tail vein, various forms of ADM equivalent to 5 mg/kg tid at 4-day intervals. The mice were sacrificed on day 13 of the ADM treatment. The tumors were excised and weighed. The blood was collected from the retro-orbital venous plexus, and AFP concentration in the serum was measured by radioimmunoassay (RIA). The therapeutic effects were also evaluated from histological observations. Murine body weights were measured at the initiation and termination of the experiment. Any change in the body weight was evaluated.

### Statistical Analyses

The statistical significance of the differences between groups was assessed with Student's *t*-test. For $P < 0.05$, differences were regarded as statistically significant.

## RESULTS

### Efficiency of ADM Entrapment in the Liposomes and Size of Liposome Particles

Conventional Lip-ADM contained negatively charged DPPA in its lipid composition in order to increase the incorporation rate of ADM in the liposomes. Although DPPA was removed from the lipid composition of both types of st.Lip-ADM, there was no remarkable change in the efficiency of ADM entrapment between the conventional Lip-ADM and the st.Lip-ADM. The ratio of ADM incorporation in the st.Lip-ADM(I) and st.Lip-ADM(II) were 7.1% and 6.0%, respectively, as compared with 6.2% in the conventional Lip-ADM. The diameters of individual liposome particles were 42.2 ± 5.0 nm (mean ±SD) in the conventional Lip-ADM, 28.9 ± 3.0 nm in the st.Lip-ADM(I), and 49.7 ± 6.0 nm in the st.Lip-ADM(II).

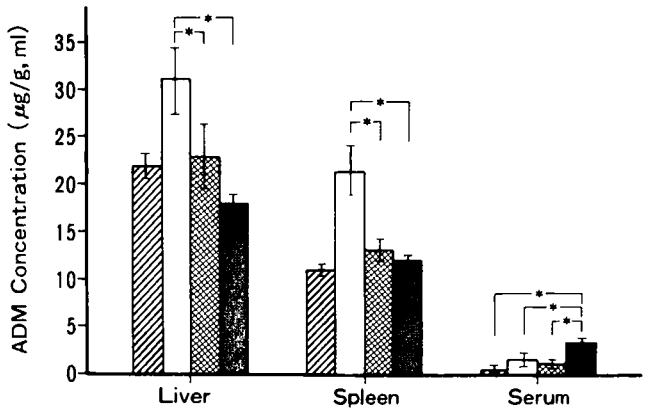


Fig. 1. Tissue distribution of Adriamycin (ADM) at 1 hr after injection in B10.BR mice. Various forms of ADM equivalent to 5 mg/kg were injected intravenously and the concentration of ADM was determined fluorometrically. Free ADM ( ▨ ), ADM encapsulated liposomes (Lip-ADM) ( □ ), type I stealth liposomes containing ADM (st.Lip-ADM(I))( ▩ ), type II stealth liposomes containing ADM (st.Lip-ADM(II)) ( ■ ). Mean ±SD. *$P < 0.05$ (n = 4).

### Tissue Distribution of ADM

B10.BR mice (n = 4 in each group) received various forms of ADM equivalent to 5 mg/kg. At 1, 4, 8 and 16 hr after the injection, ADM concentrations in various tissues were measured (experiment 1). One hr after injection, ADM concentration in the liver and spleen from the st.Lip-ADM(I) and II) groups was significantly lower than those in conventional Lip-ADM group and comparable to the levels in the free ADM group (Fig. 1). By contrast, the serum ADM level in the st.Lip-ADM(II) group was significantly higher compared with the other three groups. At 4 hr after injection, the ADM levels in the liver from the st.Lip-ADM(I) and (II) groups were still lower than those in the Lip-ADM group, although the differences were not as great as at 1hr (Fig. 2). Compared with the other three groups, a remarkably high level of ADM in the serum (2.32 ± 0.15 μg/ml) was maintained in the st.Lip-ADM(II) group at 4 hrs and even at 16 hrs after injection ADM was detectable in the serum. The serum ADM level in the st.Lip-ADM(I) group decreased slowly, so that a relatively high level of ADM (0.66 ± 0.47 μg/ml) was present at 4 hr after injection. In contrast ADM levels were 0.09 ± 0.09 μg/ml in the free ADM group and 0.53 ± 0.45 μg/ml in the Lip-ADM group, respectively (Fig. 2).

In the subsequent experiment (experiment 2), Li-7 bearing nude mice (n = 5 in each group) received various forms of ADM equivalent to 10 mg/kg, and ADM concentrations in various tissues were compared at 4 hr after injection (Fig. 3). The ADM levels in the liver and spleen from the st.Lip-ADM(I) group were significantly lower than those in the Lip-ADM group. The ADM concentra-

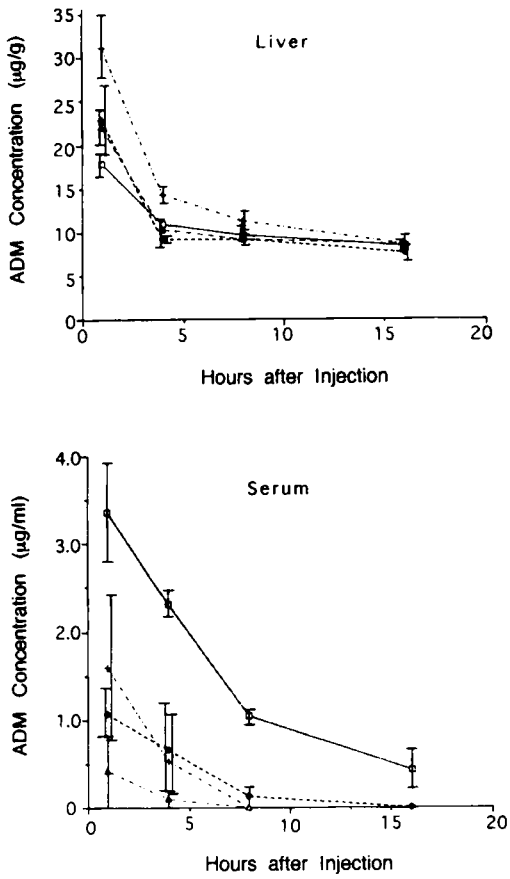


Fig. 2. Adriamycin (ADM) levels in the serum and liver at 1, 4, 8, and 16 hr after injection in B10.BR mice (ADM equivalent to 5 mg/kg i.v.). Free ADM (— △ – –), ADM in liposomes (– · + – ·), ADM in type I stealth liposomes (– – ◇ – –), ADM in type II stealth liposomes (— □ —).

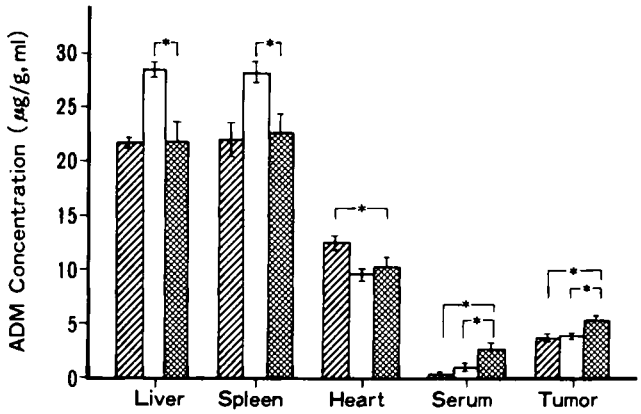


Fig. 3. Tissue distribution of Adriamycin (ADM) at 4 hr after injection in nude mice bearing hepatoma Li-7. Various forms of ADM equivalent to 10 mg/kg were injected i.v. and the concentration of ADM was determined fluorometrically. Free ADM ( ▨ ), ADM in liposomes ( □ ), ADM in type I stealth liposomes ( ▩ ). Mean ±SD. *$P < 0.05$ (n = 5).

**TABLE I. Tissue Distribution of Adriamycin: Ratio of Heart/Tumor and Liver/Tumor†**

| | Heart/tumor | Liver/tumor |
|---|---|---|
| Free ADM | 3.61 ± 0.83* | 6.29 ± 1.49* |
| Lip-ADM | 2.55 ± 0.48* | 7.69 ± 1.20* |
| Stealth Lip-ADM = (I) | 2.01 ± 0.39* | 4.31 ± 0.60* |

†Values represent mean ±SD (n = 5). Adriamycin (ADM) were administered to tumor-bearing mice as free ADM, ADM-encapsulated liposome (Lip-ADM) or ADM encapsulated in type I stealth liposome (st.Lip-ADM(I)). At 4 hr, levels of ADM in heart, liver, and tumors were determined and their heart/tumor and liver/tumor ratios calculated. *$P < 0.05$.

tion in the tumor was significantly higher in the st.Lip-ADM(I) group (5.06 ± 0.39 μg/g) than in the free ADM group (3.58 ± 0.66 μg/g) or in the Lip-ADM group (3.76 ± 0.40 μg/g). Moreover the serum ADM level was significantly higher in the st.Lip-ADM(I) group (2.52 ± 0.97 μg/ml) than in the other groups (free ADM group: 0.20 ± 0.20 μg/ml, Lip-ADM group: 0.83 ± 0.46 μg/ml). On the other hand, the ADM concentration in the heart, which poses a problem in clinical use of ADM, was slightly higher in the st.Lip-ADM(I) group (10.12 ± 1.66 μg/g) compared with the Lip-ADM group (9.39 ± 0.92 μg/g), but significantly lower compared with the free ADM group (12.43 ± 0.86 μg/g). When liver/tumor ratio and heart/tumor ratio of ADM concentrations were compared (Table I), the values of both ratios were lower in st.Lip-ADM(I) group than those in the other groups.

## Therapeutic Effect of ADM in Stealth Liposomes In Vivo

The Li-7-bearing nude mice (n = 4–5) received various forms of ADM equivalent to 5 mg/kg tid at 4-day intervals, and they were sacrificed on day 13 of the treatment. At the beginning of the ADM treatment, the mean of estimated tumor weights in each group was 243–283 mg, and there was little difference among them. On day 13 of the treatment, the excised tumor weights were less both in st.Lip-ADM(I) = Ab (203 ± 40 mg) and st.Lip-ADM(II) = Ab (174 ± 107 mg) groups than those in free ADM group (635 ± 111 mg) ($P < 0.05$) or those in Lip-ADM = Ab group (424 ± 177 mg) (although we could not conclude as significant) (Fig. 4). Furthermore, the therapeutic effect of ADM in stealth liposomes was investigated from the viewpoint of growth inhibition of tumors by measuring the estimated tumor weight at various days after initial administration. As shown in Figure 5, the effects of ADM in stealth liposomes were consistently better than those of ADM in other forms and they were much evident after the third injection.

Comparing the serum AFP levels in each group on day 13 of the treatment, there was a tendency that AFP levels were lower in st.Lip-ADM = Ab, st.Lip-ADM(I) = Ab, and st.Lip-ADM(II) = Ab groups than those in control and free ADM groups (Fig. 6).

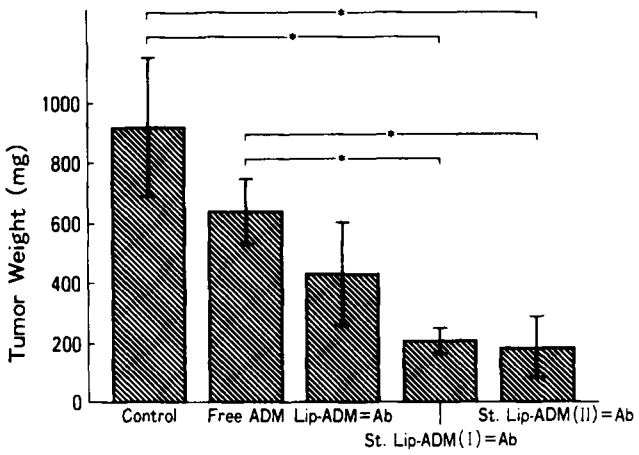


Fig. 4. Therapeutic effect of Adriamycin (ADM) administered in various forms on the growth of Li-7 inoculated on the back of nude mice. The results are expressed as mean tumor weights excised on 13th day of the treatment. Various forms of ADM equivalent to to 5 mg/kg were injected i.v. three times every 4 days. Experimental groups are saline alone (control), free ADM, ADM in 19-F-12-conjugated liposomes (Lip-ADM = Ab), ADMin 19-F-12-conjugated type I stealth liposomes (st. Lip-ADM(I) = Ab), and ADM in 19-F-12-conjugated type II stealth liposomes (st. Lip-ADM(II) = Ab). Mean ±SE. $*P < 0.05$ (n = 4–5).

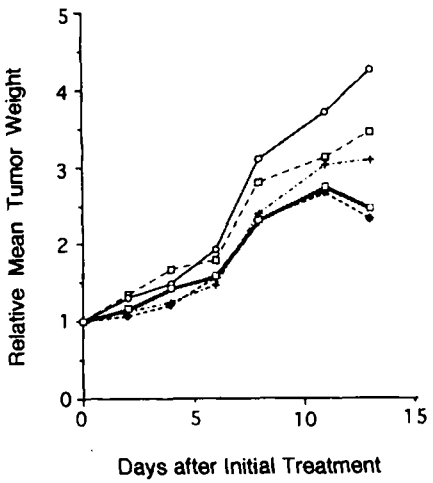


Fig. 5. Tumor growth in nude mice after treatment with various forms of Adriamycin (ADM). The results are expressed as relative mean tumor weights (Wi/Wo), where Wi is the estimated tumor weight at given time and Wo at the initiation of treatment. The estimated tumor weight was calculated as $1/2 \times \text{length} \times \text{width}^2$ of the tumor. When tumors reached about 250 mg at estimated tumor weight, three injections of saline (— ○ —), free ADM (– – □ – –), ADM in 19-F-12-conjugated liposomes (– · + – ·), ADM in 19-F-12-conjugated type I stealth liposomes (– – – ◇ – – –) or ADM in 19-F-12-conjugated type II stealth liposomes (— □ —) were administered 4 days apart.

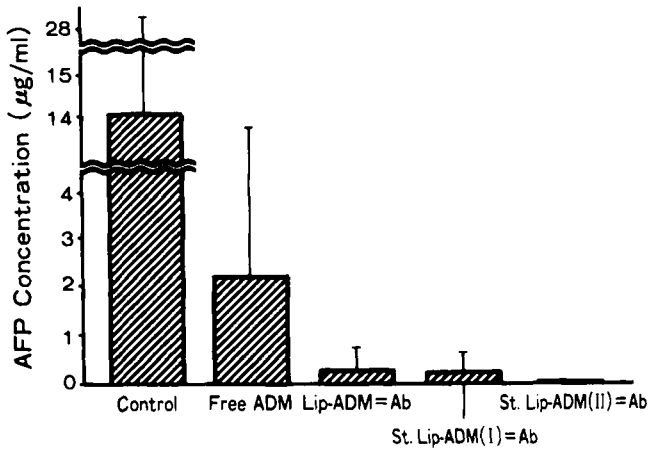


Fig. 6. α-Fetoprotein (AFP) concentrations in the serum of Li-7-bearing nude mice. Treatment groups were the same as shown in Figure 4. On 13th day of the treatment, the murine blood was collected from each group and AFP concentration in the serum was measured by RIA. Mean ±SD.

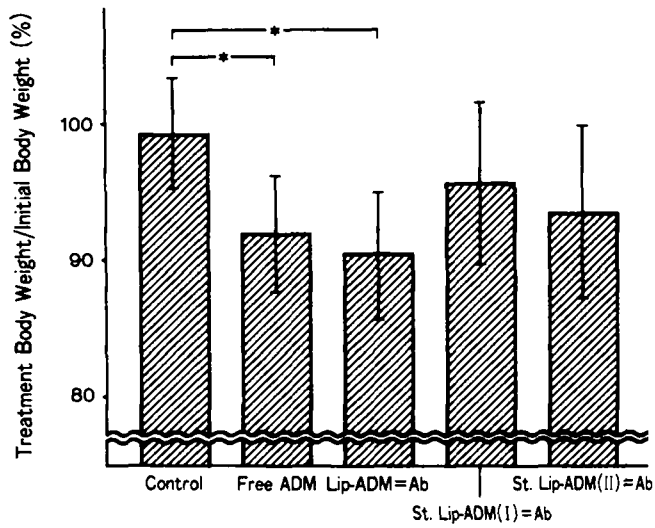


Fig. 7. Changes in body weight after treatment with various forms of Adriamycin. Treatment groups were the same as shown in Figure 4 and the results are expressed as the ratio of body weight on the 13th day of treatment to that at the initiation of treatment. Mean ± SD $*P < 0.05$.

The therapeutic effect of ADM was also confirmed by histological examinations (data not shown). In the case of free ADM and Lip-ADM = Ab groups, necrosis was observed in large part of the tumor, but viable cancer cells were seen and cancer nests remained in some parts. In the case of st.Lip-ADM(I) = Ab and st.Lip-ADM(II) = Ab groups, destruction of the cancer cells and cancer nests was marked and viable cancer cells were hardly seen.

We compared the decrease in murine body weights of each group, as a measure of drug toxicity (Fig. 7). A significant decrease in body weight was observed in free ADM and Lip-ADM = Ab groups, as compared with the control group, but the decrease was less in the st.Lip-ADM(I) = Ab and st.Lip-ADM(II) = Ab groups.

## DISCUSSION

As a drug carrier, particularly as a carrier of anticancer agents, liposomes have several advantages. They are nontoxic and biodegradable. Liposomes can enclose the drug without chemical modifications so that they can deliver the drug to the target site without changing its therapeutic effect. Furthermore reduction of the side effect (e.g., cardiotoxity of ADM) is possible by encapsulation of the drug into liposomes, and targeting chemotherapy can be performed by conjugating various kinds of antibody to the surface of liposomes. However, at the same time, liposomes have the shortcoming that they tend to be readily entrapped by the RES, causing accumulation of the anticancer agents in liver and spleen [3,8].

In order to evade the liposome uptake by the RES, Hashimoto et al. [9] tried to saturate and block the Kupffer cells and the splenic macrophages with a lot of vacant liposomes beforehand. However, it is not beneficial for the host's defense system to suppress the host's RES function even temporarily [10], and in some cases it will be dangerous. We have used small unilamellar vesicles, for the RES uptake decreases parallel to the down-sizing of liposomes, but its effectiveness is still limited. Therefore it is desirable to change the character of liposomes themselves so that they can evade entrapment by the RES, that is, making liposomes stealthy. We called these liposomes stealth liposomes because of their reduced uptake by RES. In this study, we attempted to give stealthy character to the conventional Lip-ADM by adding ganglioside GM1 to the lipid composition of the liposome, and evaluated the possibility of pharmacokinetic improvement and reinforcement of therapeutic effect.

GM1 is a glycolipid that exists in brain and leukocytes of human and other animals [11,12]. GM1 has attracted attention since Allen and Chonn [13] and Papahadjopoulos and Gabizon [14,15] reported that the addition of GM1 to the lipids composing liposomes reduced the uptake of liposomes by the RES and prolonged the lifetime of liposomes in the blood circulation. However, at this moment it is unclear through what mechanism GM1 helps the liposomes to escape the uptake by the RES. Looking at molecular structure of GM1, one molecule of N-acetylneuramic acid (NANA), a form of sialic acid, and ceramide, bind to carbohydrate chain composed of glucose, galactose, and N-acetylgalactosamine [11,12]. It is known that GM1 has a much stronger stealth effect than other members of the ganglioside group, and asialo-GM1, in which NANA is removed from GM1, has only a weak stealth effect [13,16]. This suggests that the number of NANA and its position in the molecule may play an important role in its mechanism. Allen et al. [17] speculated the following mechanism. GM1 has a negative charge in the NANA area, and this prevents aggregation of liposomes. Furthermore, because the negative charge of GM1 is not exposed to the liposome surface, it evades opsonization in blood, so that liposomes are not easily recognized by Kupffer cells and macrophages. Indeed, in the case of phosphatidylserine that is also negatively charged but the negative charge is exposed to the surface, the presence of this lipid made liposomes readily captured by the RES, contrary to GM1 [16]. However, Mori et al. [18] reported that the stealth activity of GM1 did not correlate with its steric barrier activity on the liposome surface, and the role of GM1 in stealth liposomes is still unclear.

In this study, we prepared two types of stealth liposomes. In addition to type I, we prepared type II by replacing one-half of egg PC with SM in order to prolong the lifetime of liposomes in the blood circulation, as the addition of SM to liposome lipids is known to increase the rigidity of liposomes [17]. Since our preliminary experiment had shown that addition of DPPA to stealth liposome resulted in loss of this property, DPPA was removed from both types of stealth liposomes. Also in a preliminary experiment, we prepared liposomes in which one half of egg PC was replaced with SM but without GM1, and evaluated its pharmacokinetics in tumor-free mice. However, the stealth effect was not observed with such liposomes.

Our experiments demonstrated here that antibody-linked stealth liposomes had a high antitumor effect, but a number of problems have to be cleared before the clinical application of this drug delivery system. First, although the serum level of ADM increased with stealth liposomes, the transfer of ADM in the liposomes to the tumor may vary considerably depending on the types of tumors and the properties of liposomes. In accordance with our results, an efficient targeting effect of antibody-linked stealth liposomes has been reported with the system of murine thymoma cells [14] and lung endothelial cell [19]. However, in our preliminary experiments with a human gastric carcinoma cell line MKN-45, the stealth liposomes produced little elevation of ADM concentration in the tumor (data not shown). This may be attributed to the differences in the structure of blood vessels and vascular permeability in tumor tissue [20]. Furthermore, the types of stealth liposomes will influence the transfer of drugs into tumor cells. When ADM encapsulated in liposomes enters into tumor cells by endocytosis of liposomes, the more stable they are, the more ADM enters into the target cells. However, when ADM enters into the tumor cells mainly by passive diffusion of ADM after extracellular disintegration of liposomes, too stable liposomes such as type(II) liposomes could work negatively on the delivery of ADM to the tumor. Therefore, the merit of using the stealth liposomes might differ from tumor to tumor, but for tumors with abundant blood flow and with a property of endocytosing liposomes, targeting of

drugs to the tumor will be greatly enhanced by making liposomes stealthy.

Second, the cost of GM1 is extremely high. Since the amounts of GM1 required for repeated clinical use are far greater than that used in our experiment, it would cost enormously. Therefore, to apply it clinically, it is essential to lower the cost by improving the method of GM1 extraction or synthesis. In recent years, attempts have been made to use hydrogenated phosphatidylinositol [21,22], polyethyleneglycol [23–26], or palmityl-D-glucuronide [27], which are more easily obtained than GM1, to give liposomes a stealth effect.

The third problem is the antigenicity of antibody. Since monoclonal antibody used in our experiment is derived from the mouse, it produces human antimouse antibody when used clinically [28]. As a solution to this problem, some modification of the antibody should be considered, such as chimera antibody obtained by replacing a highly antigenic Fc fragment with human type [29], or Fab′ antibody obtained by removing an Fc fragment [30].

The decreases in body weight during treatment in the stealth liposome groups tended to be smaller compared with the other groups, and this point should also be evaluated. However, since inhibition of tumor growth might have alleviated emaciation of mice, it is unclear how much the decreased ADM accumulation in the liver played a role in the body weight change in mice.

In conclusion, the stealth liposomes were sufficiently effective in reducing the accumulation of Lip-ADM in the liver and splenic tissues. In terms of the ability to promote the transfer of ADM to the tumor and to increase the antitumor effect, the stealth liposomes have several problems as discussed above. Nevertheless, these liposomes are expected to be effective in some types of tumors such as hepatoma.

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